Docket No.: PBLI-P01-007
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sidney Pestka

Application No.: 09/872,349

Filed: May 31, 2001

For: PHOSPHORYLATED PROTEINS AND USES RELATED THERETO

Confirmation No.: 1152

Group Art Unit: 1642

Examiner: L. R. Helms

DECLARATION UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Mark Walter, Ph.D., of Birmingham, AL, hereby declare as follows:

1. I am an Associate Professor in the Department of Microbiology, University of Alabama at Birmingham. A copy of my curriculum vitae is attached hereto as Exhibit A.

2. I have read the Office Action issued by the United States Patent and Trademark Office on September 13, 2004 (the "Office Action"). I have also read the specification of U.S. Application Serial No. 09/872,349 (the "subject application") and the claims pending in the Office Action.

3. I understand that the subject application was filed on May 31, 2001 and claims priority to May 31, 2000.

4. I understand that the claimed invention relates to a phosphorylatable antibody or antigen binding fragment thereof, engineered to include at least one heterologous kinase recognition site located in the hinge region and which does not adversely affect the ability of the antibody or antigen binding fragment to bind antigen, such that an added phosphate group of a phosphorylated form of the antibody or antigen binding fragment is protected from hydrolysis by intramolecular interactions with other amino acid residues so that at least 80% of all the phosphate groups remain attached after at least 5 days of incubation of the phosphorylated form in animal serum or buffer.

5. I understand that the Examiner has alleged that the subject patent application is enabling only for antibodies with "a kinase site at position 224 or at the C-terminus in the linear amino acid sequence." I further understand that the Examiner alleged that the "specification does not enable antibodies with a kinase site other than position 224 with the claimed properties." *See* page 4 of Sept 13, 2004 Office Action ("Office Action").

6. Based on the teachings of the subject application and what I knew as of May 31, 2000, I would have been able to make the claimed invention without undue experimentation. That is, I would have been able to make an antibody with a kinase recognition site at a position other than serine 224 or in the carboxy-terminus, such as, in the hinge region.

7. By May 31, 2000, molecular modeling of monoclonal antibodies and antibody fragments was practiced by numerous investigators in the field. Included in Exhibit B is a list of nine relevant articles covering modeling of monoclonal antibodies authored by investigators from at least nine different research laboratories worldwide. The articles were published between January 1, 2000 and May 31, 2000. There are a number of additional examples published prior to 2000. Furthermore, the literature covering molecular modeling of proteins in general is extremely extensive as of this date. Selected reviews covering the expanse of molecular modeling of proteins and published prior to May 31, 2000 include those by Eisenhaber *et al.*, Reithmeier, Bohm, and Moult (Eisenhaber *et al.*, 1995; Reithmeier, 1995; Bohm, 1996; Moult 1996; Moult, 1999).

8. The working examples and modeling methods described by the inventor in the specification allow one skilled in the art to adopt the methodology to scan the hinge region and, further, the entire monoclonal antibody backbone, for those locations where phosphoserine or other phospho-amino acid stability is favored according to the guideline of the specification. The method of arriving at the specific examples included in the specification and the inclusion of these examples are sufficiently described to enable the application of the method 1) to insert a kinase site at any region of the molecule, and 2) to examine the local interactions of the phosphate on the phosphoserine residue. The further characterization of the monoclonal antibodies first through expression of monoclonal antibodies, which was practiced worldwide, and then through stability experiments, which were described in and enabled by the specification, were commonplace and not unduly difficult. The working examples and methodology described enable the broader practice of the method over the entire molecule. Full citations for references cited herein are listed in Exhibit C.

9. To elaborate further on the enablement provided, it is useful to include the pending claim 1 as of June 24, 2004, which recites as follows:

> A phosphorylatable antibody or antigen binding fragment thereof, engineered to include at least one heterologous kinase recognition site located in the hinge region and which does not adversely affect the ability of the antibody or antigen binding fragment to bind antigen, such that an added phosphate group of a phosphorylated form of the antibody or antigen binding fragment is protected from hydrolysis by intramolecular interactions with other amino acid residues so that at least 80% of all the

phosphate groups remain attached after at least 5 days of incubation in vitro of the phosphorylated form in animal serum or buffer.

10. *Molecular modeling:* The first portion of this claim addresses the location of the heterologous kinase site, specified in claim 1 to be the hinge region. The examiner does not dispute that position 224 is contained within the hinge region. The concern of the examiner rests with whether the example of phosphorylation at position 224 sufficiently enables a claim reciting the entire hinge region. The inventor describes a molecular modeling approach in the specification to facilitate identification of sites where phospho-amino acid stability is favored. The stated modeling method could reasonably have been practiced by those skilled in the art as of May 31, 2000. The specific technique described by the inventor is one of homology modeling, well known and practiced in the field for some time (Greer 1981; Greer, 1990) with larger protein structure databases further facilitating this strategy (Bernstein, *et al.*, 1985; Burks *et al.*, 1985). From the relevant art practiced and published by May 31, 2000, and as included in Exhibit B, it is clear that molecular modeling of monoclonal antibodies was practiced by numerous investigators skilled in the art. The software packages utilized by the inventor were commonly available by May 31, 2000, and the crystal structure of the template monoclonal antibody, MAb231, was published in 1992 (Harris *et al.*, 1992). The practice as described in the specification could have been applied without undue burden to modeling of other residues in the hinge region (and beyond) to determine whether these residues would similarly serve as suitable sites for phosphate addition and stabilization. In particular, I draw the distinction here between crystallizing a protein and performing homology modeling of a protein based on a crystal structure. The examiner cites the difficulty of obtaining a crystal structure of a monoclonal antibody in Paragraph 8, Response to Arguments. At the time of this application, this was undoubtedly true. However, the application of homology modeling to a monoclonal antibody of unknown structure *once the first crystal structure was published* was not unduly difficult as the published monoclonal antibody serves as the template for the modeling effort. The inventor does not adopt a crystallization approach but takes advantage of the time-saving approach of homology modeling to predict appropriate sites for kinase site insertion.

11. It is clear that any residue in the hinge region or, for that matter, throughout the entire protein, can be homology modeled in a similar fashion using the methodology adopted and described by the inventor. In fact, once a template structure became available in 1992, and once a single monoclonal antibody was modeled by the inventor using the published crystal structure coordinates, further changing of the input (unknown monoclonal antibody) sequence by a few amino acid residues in that sequence is fairly trivial and can be executed by someone possessing basic skill in the art. Homology modeling efforts of this nature could easily have been executed in rapid succession for a variety of residues in the hinge region and beyond. The iterative process of scanning all residues in the hinge region by this method for suitability for phosphorylation would not have been unduly difficult to execute. Table 1 of the specification clearly indicates that the procedure was applied to the examination of other residues in the monoclonal backbone.

12. As mentioned above, the inventor describes the use of the methodology in the development of a monoclonal antibody modified in the local region of SER 224, thus meeting the requirement of providing the best available example of the art in the application. Again, this description would enable a suitably skilled practician to apply this method broadly to other sites including those in the hinge region. Performing this modeling approach for kinase recognition sequences larger than a few amino acids, such as those of casein kinase I, casein kinase II, and *src* tyrosine kinase, would be similarly straightforward, and is enabled by the methodology provided in the application. The inclusion of the molecular modeling approach by the inventor is expressly intended to *reduce* the experimentation effort required to develop monoclonal antibodies with stable phosphorylation sites that would be necessary from pure guesswork or random searching. Though the practice of molecular modeling requires technical training, the intended outcome of the inventor's application of molecular modeling is to provide a much more direct approach to developing potentially successful therapeutic molecules than characterization of randomly chosen phosphorylation sites throughout the backbone of the antibody heavy chain. The molecular modeling approach clearly focuses the effort on those amino acid regions that fit the modeling criteria specified by the inventor. As demonstrated by the inventor in the hinge region example provided, once the homology modeling approach yielded a site of interest that appears to foster increased phosphate stability, the remaining work of producing and testing the molecule was straightforward and able to be practiced widely in the biomedical community. The further application of homology modeling to sites nearby to position 224 is clearly facilitated by the teachings of the application and the working example provided therein.

13. *Monoclonal antibody construct synthesis, transfection, expression, and purification:* Within the body of the application, the inventor specifies a molecular modeling-based approach to develop candidate sites where phosphate addition could be expected to result in low rate of hydrolytic release from the target tyrosine, threonine, serine, or other amino acid after enzymatic phosphorylation. Subsequent to the modeling approach, the inventor produces at least one working example of such a molecule, capable of being phosphorylated by cAMP-dependent kinase and demonstrating excellent phosphate stability. The inventor describes example procedures for producing such a molecule through methods common in the art, *i.e.*, genetic engineering, cell transfection, protein expression, and protein purification. Specifically, the inventor first specifies the straightforward genetic engineering approach of site-directed mutagenesis by which a specific DNA sequence, corresponding to the cAMP-dependent kinase consensus target sequence, is inserted into the hinge region of the heavy chain of the monoclonal antibody ssDNA, and the heavy chain clone containing the kinase site was subsequently inserted into a plasmid (Figures 18 to 26 and accompanying text). Upon generation of the appropriate heavy chain cDNA clone and confirmation of the DNA sequence by conventional means, the inventor then, again through the widely practiced means of electroporation, inserted the DNA sequence in an expression vector (Figure 17) into a mouse myeloma (NSO) cell line. The methods for selection of a cell clone with strong expression of a monoclonal antibody in the cell culture media were widely practiced. Expression of the monoclonal antibody in the cell culture fluid by a high expressing NSO cell clone was executed by standard cell culture techniques. Purification of the example phosphorylatable monoclonal antibody is also described in the specification and was

carried out through standard chromatography practices (Paragraphs 0146 and 0147). This entire procedure is straightforward and calls for no undue experimentation, particularly for one skilled in the art, in order to execute the procedure for multiple monoclonal antibodies, each bearing a unique phosphorylation site within the hinge region. Again, the homology modeling approach is intended to reduce the experimentation required to achieve a monoclonal antibody exhibiting the desired phosphate stability.

14. *Phosphorylation reaction:* The phosphorylation reaction described in the specification follows conventional procedures for transfer of the γ-phosphate of ATP (or another nucleoside triphosphate such as GTP) to the acceptor amino acid of a protein substrate (Paragraph 0148). The phosphorylatable monoclonal antibody was labeled with ^{32}P-phosphate with the use of γ-^{32}P-ATP and the cAMP-dependent protein kinase, and the incorporation of radioactivity into the protein was measured with a liquid scintillation spectrometer after precipitation of the protein with trichloroacetic acid. Each of these methods was published prior to May 31, 2000 (Lin, *et al.*, 1996; Lin, *et al.*, 1998).

15. *Effect on antigen binding:* Since it was known in the art that the antigen binding site of the variable domain was functional and effective even when removed from the rest of the antibody or when the antibody was modified significantly (single chain antibodies - scFv; removal of CH2 domain; use of various fragments of antibodies for many years such as Fab, Fab2 fragments), it was known in the art that significant modifications of the antibodies could be made without significant alteration of their affinities for antigen. These modified antibodies and antibody fragments were used very effectively in many assays: immunoprecipitation; Western blotting; ELISA assays; etc. The changes described by the inventor are small in comparison to these other changes and not likely to change the affinity of the antibody as long as the complementarity determining regions (CDR) of the antibody are not compromised. As the CDR is only a small region of the entire antibody and is distal to the hinge region, modifications to the hinge region are highly unlikely to alter affinity of the resulting molecule for antigen.

16. Chimeric antibodies have been routinely produced without significant change to the affinity. It has been possible to effectively graft the relatively small CDR region from a mouse antibody into a human antibody framework. It was found that not all residues of the CDR were necessary to maintain affinity of the antibody. For example, only 20-33% of the CDR residues might be involved in the Ag-Ab interaction (Padlan, 1994). These residues, designated as specificity determining residues (SDRs), can be located with the aid of the three-dimensional structure of the MAb and the genetic analysis of the Ag binding site. Humanization of a murine MAb by grafting only SDRs into the framework of a human MAb could reduce the immunogenic potential of a murine MAb to a minimum (Iwahashi *et al.*, 1999; Tamura *et al.*, 2000 - Feb. 1, 2000) while retaining antigen specificity and affinity. Thus, there is ample evidence that major changes in antibodies have relatively little effect on the affinity. The inventor is correct that changes introduced by the engineering of a phosphokinase binding site would have little effect on antibody affinity for antigen.

17. The direct binding assay utilized by the inventor (Paragraphs 0149 and 0150) follows strategy published previously by others in the field (Schott *et al.*, 1992; Slavin-Chiorini *et al.*, 1995) with the further modification of coating the target proteins onto beads (Johnson *et al.*, 1986; Kashmiri *et al.*, 1995). These techniques can be practiced by one of ordinary skill in the art.

18. I conclude that practitioners of reasonable skill in the art of molecular modeling, standard molecular biology, and biochemical methods would have been sufficiently enabled by the descriptions provided in the specification to execute similar studies without undue burden and could have done so in an iterative manner. Both working examples provided (*e.g.*, the hinge region-modified monoclonal antibody) and the teachings and means by which this example was obtained enable the practice of the method and the application to other residues in the hinge region, and beyond. The combination of molecular modeling with standard biology and biochemistry employed by the inventor is clearly aimed at reducing the burden of experimentation, and the provision of a successful example of the strategy facilitates its further practice. I or others in the field could have adopted the strategy to assess other sites in the hinge region as potentially excellent locales for the insertion of a phosphorylation site; furthermore, the strategy could easily be used to delineate virtually any other region in the monoclonal antibody outside the CDR for the insertion of a phosphorylation site.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Mark Walter, PhD

7-11-05
Date

Exhibit A

CIRRICULUM VITEA

Mark R. Walter, Ph.D.

PERSONAL INFORMATION

Title:	Associate Professor of Microbiology and Structural Biology Program Director U.A.B. Comprehensive Cancer Center
Business Address:	Room 144, Center for Biophysical Sciences and Engineering 1025 18th Street South Birmingham, AL 35294
Business Phone:	(205)934-9279
FAX:	(205)934-0480
Email:	walter@uab.edu
Web page:	http://torus.cbse.uab.edu/walter/
Home Address:	3531 East Street Birmingham, AL 35243
Phone:	(205) 970-0374
Date of Birth:	9-24-1962
Place of Birth:	Orange, California
Citizenship:	U.S.A.
Marital Status:	Married, 1-29-94, Leigh J. Walter
Children:	Hannah Claire (2-18-97) and Samuel Mark (1-05-00)

EDUCATION

High School:

1980	El Modena High School, Orange, CA

University:

1984	B.S. (Summa Cum Laude), Chemistry with Biology Minor. California Lutheran University, Thousand Oaks, CA
1989	Ph.D. Biochemistry, Lab of Charles E. Bugg University of Alabama at Birmingham, Birmingham, AL

Postdoctoral Training:

1989-1991	Lab of Steven E. Ealick University of Alabama at Birmingham Birmingham, AL

PROFESSIONAL EXPERIENCE

Academic Appointments:

1991-92	Senior Research Associate	Center for Macromolecular Crystallography, University of Alabama at Birmingham
1992-97	Assistant Professor	Department of Pharmacology University of Alabama at Birmingham
1992-	Associate Scientist	Comprehensive Cancer Center University of Alabama at Birmingham
1995-	Associate Scientist	Cell Adhesion and Matrix Research Center, University of Alabama at Birmingham
1997-	Secondary Appointment	Department of Biochemistry and Molecular Genetics University of Alabama at Birmingham
1997-00	Assistant Professor	Department of Microbiology, University of Alabama at Birmingham
1998-	Director, Structural Biology Program	Comprehensive Cancer Center University of Alabama at Birmingham
2000-	Associate Professor	Department of Microbiology, University of Alabama at Birmingham.
2003-	Secondary Appointment	Department of Physiology and Biophysics, University of Alabama at Birmingham

Professional Consultantships:

1994-01	Schering Plough Research Institute, Kenilworth, NJ
1997-98	Canji Inc., San Diego, CA
1998-01	DNAX Research Institute, Palo Alto, CA
2000	Fish & Neave, New York, New York

Awards and Honors:

1983	ACF-PRF Research Fellow, University of Wyoming, Laramie, WY
1996	Presidential Early Career Award, NIAID (1 of 10 from all NIH divisions)
2000	ISICR International Travel Award

Professional Societies:

Federation of American Societies for Experimental Biology (FASEB)
International Society for Interferon and Cytokine Research (ISICR)
Biophysical Society
Protein Society

PROFESSIONAL EXPERIENCE (continued)

Memberships:

American Crystallographic Association
American Association for the Advancement of Science

Study Sections:

1999	National Multiple Sclerosis Society
1999-2000	Israel Science Foundation
2001	NIH BBCB
2002-2005	NIH F04B Biochemical Biophysical Sciences
2004	Microscopic Imaging (MI)
2004	NIH/NCI Cancer Center Site Visitor - Duke
2005	NIH/NCI Cancer Center Site Visitor - University of Colorado

Organization of Scientific Meetings:

1996	Co-chairman, Receptors and Signal Transduction Microsymposia, International Union of Crystallography meeting, Seattle, WA
1998-	Organizer, UAB Structure-Function Seminar Series
1999	Program Chair, Cell Surface Receptors/Interactions. West Coast Protein Crystallography meeting, Asilomar, CA
2000	Session Chair, Crystallization of membrane proteins, 8th International conference on the Crystallization of Biological Molecules, San Destin FL
2004	International Advisory Board, 2005 ISICR Meeting, Bejing China

Recent Abstracts:

2001	"The Structure of an IL-10 monomer/anti- IL10 Fab Complex and the Structural Basis of Cytokine Neutralization" American Crystallographic Association, Los Angeles, CA
2001	"Structure of Human Cytomegalovirus IL-10 Bound to Soluble Human IL-10R1" International Society for Interferon and Cytokine Research, Turin Italy
2002	"Crystal Structure of Human Cytomegalovirus IL-10 Bound to Soluble Human IL-10R1". Protein Society Meeting, San Diego, CA.
2004	"N-linked Glycosylation and Conformation Changes Mediate the Low Affinity IL-22/IL-10R2 Interaction" Keystone Symposia in Structural Biology, Snowbird, Utah.
2004	"The M-PMV Gag matrix domain modulates and early stage of viral release Retroviruses", Cold Spring Harbor, New York.
2004	"The IL-10R2 binding site on IL-22 is located on the N-terminal helix and is dependent on N54 glycosylation". ASBMB Boston, MA.
2004	"Structure and Thermodynamic Analysis of the Interaction between Epstein-Barr Virus IL-10 and IL-10R1". American Crystallographic Association, Chicago, Illinois.

PROFESSIONAL EXPERIENCE (continued)

2004 "IL-22 Can Enhance Activity of IFN-γ through the IFN-γ receptor chains. Cytokines in Immunity and Cancer. International Society for Cytokine Research Meeting, San Juan Puerto Rico.

Councils and Committees:

1995-97	Graduate Committee Pharmacology
1998-99	Department of Microbiology Virology Faculty Search Committee Member
1998-	Department of Microbiology Faculty Development Committee
1997-	School of Medicine Structural Biology Committee
2000	Department of Microbiology, David Wells Award Committee
2002-03	Cellular and Molecular Biology (CMB) Graduate Admissions Committee
2002	Department of Microbiology Virology Search Committee Member
2003	Microbiology Graduate Recruitment Committee
2003	Executive Committee, Center for Integrative Structural Biology
2003	Advisory Board Member, UAB Cancer Center X-ray Core Facility
2004-2005	Department of Microbiology Virology Faculty Search Committee Member

Teaching:

1990-	Lecturer, Advanced X-ray Crystallography
1995-97	Lecturer, Pharmacology I
1996-	Course Master, Journal Club in Biological Crystallography
1996	Co-Course Master, Pharmacology I
1998	Lecturer and material development for the continuing education video "Recombinant Alfa Interferon from Naturally Occurring Genes", Applied Clinical Communications Parsippany, NJ
1998-	Lecturer, Protein Structure component of CMB I
1998-	Lecturer, Advanced Protein Structure
1999	Lecturer, Molecular Biology of Cell Adhesion
2000	Lecturer, Biochemistry / CMB Laboratory Tutorial Daulphin Island, AL
2003-	Lecturer, CMB Laboratory Methods Class
2004-	Course Master, CMBVII Molecular Recognition and 3D Molecular Graphics

Reviewer (Adhoc) for Scientific Journals:

Acta. Cryst. D., F.
Biochemistry
Biochimica et Biophysica Acta
International Immunology
Journal of Biological Chemistry
Journal of Immunology
Journal of Molecular Biology
Nature Structural Biology
Protein Science
Science

Patents:

Crystallization of IL-10, Filed 1995, Case JB0568

GRANT SUPPORT

Past Support:

Crystallization and Structure Determination of Interferon-γ Receptor and Complex, Pharmaceutical Manufacturers of America Foundation. P.I. M.R Walter,1-93 / 12-94, $25,000

Structural Studies of the HIV-1 Rev-RRE Interaction, Center for Aids Research, UAB. P.I. M.R. Walter, 11-1-93 / 2-28-95. $15,000.

Structural Studies of the Interferon-γ Receptor Complex, American Cancer Society Jr. Faculty Development Grant. P.I. M.R.Walter 3-21-94 / 2-28-95. $16,570.

Structural Basis for receptor recognition by interferon alpha and interleukin 10, American Heart Association, P.I.- M.R. Walter, 7-1-96/6-30-99. $131,379

Structural Studies of Immune Receptors, Schering Plough Research Institute. P.I. M.R. Walter 7-1-96 / 6-30-00. $700,000

Hitchings-Elion Fellowship, Burroughs Wellcome Fund Principle Investigator- M.R.Walter, Trainee- Perry Kirkham 9-1-97/2-28-99. $70,774

Crystallographic Studies of Acetyl Transferases, Schering Plough Research Institute. P.I. M.R.Walter 3-1-98/2-28-00. $300,000

Molecular Immunopathogenesis of Demylenating Disease, P01, NIH, PI John Whitaker 4-1-96 / 3-31-2001. $5,080,212.

Structural Studies of the Interferon-gamma Receptor Complex, FIRST, NIH P.I. M. R. Walter, 4-1-96 / 3-31-2001. $503,901.

Structure and Function of KLF4. Cancer Center Structural Biology Internal Award. 6-02 / 12-03. $50,000

Current Support:

Structure Studies on Proteins that Modulate IL-10 Action, RO1AI047300-05, NIH/NIAID
P.I., M.R. Walter, 1-01-00 / 12-31-05.

Cancer Center Core Grant, P30CA013148-33, NCI,
P.I., P. Emmanuel, 3-31-05 / 2-28-10

Immunity to Bacillus Anthracis: Spore-Host Interactions, PO1 AI57599-01, NIH/NIAID,
P.I. J. Kearny, 7-1-04 / 6-31-09.

PUBLICATIONS

1. Jaeger, D.A., Finley, C.T., Walter, M.R., and Martin, C.A. (1986) Preparation and Characterization of Base Sensitive Destructible Surfactants. *J. Org. Chem.* 51, 3956-3959.
2. Ealick, S.E., Walter, M.R., and Cook, W.J. (1987) Structural Studies of *E. coli* purine nucleoside phosphorylase, *Daresbury Laboratories Review*.
3. Walter, M.R., Cook, W.J., Cole, L.B., Short, S.A., Koszalka, G.W., Krenitsky, T.A., Ealick, S.E. (1990) Three-Dimensional Structure of Thymidine Phosphorylase from *Escherichia coli* at 2.8Å Resolution. *J. Biol. Chem.* 265, 14016-14022.

4. Walter, M.R., Cook, W.J., Ealick, S.E., Nagabhushan, T.L., Trotta, P.P., and Bugg, C.E. (1992) Three-dimensional Structure of Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor. *J. Molec. Bio.* 224, 1075-1085.
5. Walter, M.R., Cook, W.J., Zhao, B.G., Cameron, R., Ealick, S.E., Walter, R.L., Nagabhushan, T.L., Trotta, P.P., and Bugg, C.E. (1992) Crystal Structure of Recombinant Human Interleukin-4. *J. Biol. Chem.* 267, 20372-20376.
6. Smith, L.J., Redfield, C., Smith, R., Dobson, C.M., Clore, M.G., Gronenborn, A.M., Walter, M.R., Nagabhushan, T.L., and Wlodawer A. (1994) Comparison of Four Independently Determined Structures of Human Recombinant Interleukin-4. *Nature. Struc. Biol.* 1, 301-310.
7. Cook, W.J., Walter, L.J., & Walter, M.R. (1994) Drug Binding by Calmodulin: Crystal Structure of a Calmodulin-Trifluoperazine Complex. *Biochemistry* 33, 15259-15265.
8. Cook, W.J., Windsor, W.T., Murgola, N.J., Tindall, Nagabhushan, T.L., & Walter, M.R. (1995) Crystallization and Preliminary X-ray Investigation of Recombinant Human Interleukin 10. *Proteins Structure Function, and Genetics* 22, 187-190.
9. Walter, M.R., Windsor, W.T., Nagabhushan, T.L., Lundell, D.J., Lunn, C.A., Zauodny, P.J., & Narula, S.K. (1995) Crystal Structure of a complex between interferon-γ and its soluble high affinity receptor. *Nature* 376, 230-235.
10. Walter, M.R, & Nagabhushan, T.L. (1995) Crystal Structure of Interleukin 10 reveals an Interferon-γ like fold. *Biochemistry* 34, 12118-12125.
11. Windsor, W.T., Walter, L.J., Syto, R., Cook, W.J., Nagabhushan, T.L., and Walter, M.R. (1996) Purification and crystallization of a co-complex between the interferon-γ receptor (extracellular domain) and interferon-γ. *Proteins Structure Function, and Genetics* 26, 108-114.
12. Ramaswamy R., Walter, L.J., Hruza, A., Reichert, P. Trotta, Nagabhushan, T.L., and Walter, M.R. (1996) Zinc mediated dimer of human interferon-α_{2b} revealed by X-ray crystallography. *Structure* 4, 1453-1463.
13. Walter, M.R. (1997) Three-dimensional models of human interferon-α subtypes IFN-α_{con}, IFN-α_8, and IFN-α_1 derived from the crystal structure of IFN-α_{2b}. *Seminars in Oncology* 24, 52-62.
14. Pfeffer, L.M., Dinarello, C.A., Herberman, R.B., Williams, B.R.G., Borden, E.C., Bordens, R., Walter, M.R., Nagabhushan, T.L., Trotta, P.P., and Pestka, S. (1997) Biological properties and clinical applications of recombinant interferons: 40th Anniversary of the discovery of interferon. *Cancer Research* 58, 2489-2499.
15. Walter, M.R., Bordens, R, Nagabhushan, T.L., Williams, B.R.G., Herberman, R.B., Dinarello, C.A., Borden, E.C., Trotta, P.P., Pestka, S., and Pfeffer, L.M. (1997) Review of recent developments in the molecular characterization of recombinant alpha interferons on the 40th anniversary of the discovery of interferon. *Can. Biother. and RadioPharm.* 13, 143-154.
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17. Ramaswamy R., Walter, L.J., Subaranian, P., Johnson, H., and Walter, M.R. (1999) Crystal structure of Ovine IFN-τ at 2.1Å resolution. *J. Molec. Biol.* 1, 151-162.
18. Josephson, K., DiGiacomo, R., Indelicato, S.R., Ayo, A., Nagabhushan, T.L, Parker, M.H., and Walter, M.R. (2000) Design and Analysis of an Engineered Human Interleukin-10 Monomer. *J. Biol. Chem.* 275, 13552-13557.

PUBLICATIONS (continued)

19. Landar, A., Curry, B., Parker, M.H., DiGiacomo, R., Indelicato, S.R., T.L. Nagabhushan, Rizzi, G., and Walter, M.R. (2000) Design, Characterization, and Structure of a Biologically Active Single Chain Mutant of Human IFN-γ. *J. Molec. Biol.* 299, 169-179.
20. Subramaniam PS, Larkin III J, Mujtaba MG, Walter M.R., and Johnson HM. (2000) The COOH-terminal nuclear localization sequence of interferon γ regulates STAT1 nuclear t ranslocation at an intracellular site. *J. Cell Sci.*113, 2771-2781.
21. Josephson, K., Logsdon, N., and Walter, M.R. (2001) Crystal Structure of the IL-10/IL-10R1 Complex Reveals a Shared Receptor Binding Site. *Immunity* 15, 35-46.
22. Josephson, K., McPherson, D.T., and Walter, M.R. (2001) Purification, Crystallization, and Preliminary X-ray Diffraction of a Complex between IL-10 and soluble IL-10R1. *Acta Cryst D* 57, 1908-1911.
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24. Josephson, K., Jones, B.C., DiGiacomo, R., Indelicato, S.R., and Walter, M.R. (2002) A Non-Competitive anti-IL-10 Neutralizing Antibody Revealed by Protein Engineering and X-ray Crystallography. *Structure* 10, 981-987.
25. Walter, M.R. (2002) Crystal Structures of α-helical Cytokine Receptor Complexes: We've Only Scratched the Surface. Biotechniques S46, 50-57.
26. Walter, M.R. (2002) Structure of the IL-10/IL-10R1 complex: A Paradigm for Class 2 Cytokine activation in *Immunologic Research* (Julius M. Cruse, Editor) *Humana Press* 26, 303-308.
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